B1

coding region; exons 1-3 (E1-3) are as indicated. Segments of Primer 3 (SEQ ID NO: 4), PPI-2 (SEQ ID NO: 5), and Primer 1 (SEQ ID NO: 6) are also shown.--

Replace the paragraph beginning at page 20, line 17 with the following rewritten paragraph:

B2

--The POMC-Insulin transgene consisted of the POMC promoter region linked to the structural region of the mouse preproinsulin II (Ins) gene (Fig 2). To excise the 5' regulatory region of the Ins gene yet preserve the translation initiation start site at position 1132, a novel Hind III restriction site was created at position 985 by site-directed mutagenesis using the recombination polymerase chain reaction (PCR) technique (Jones, D.H., Sakamoto, K., Vorce, R.L. & Howard, B.H. (1990) *Nature (London)* **344**, 793-794). A 2.4 Kb genomic Bam HI Ins fragment (Wentworth, B.M., Schaefer, I.M., Villa-Komaroff, L. & Chirgwin, J.M. (1986) *J. Mol. Evol.* **23**, 305-312) was cloned into pBluescript (pBS, Stratagene). The recombinant Ins-pBS vector was linearized in two separate restriction enzyme digestion reactions with Bal I (position 846) and PfiM I (position 1237). These templates were then amplified in two separate PCR reactions using primer 3: 5'-*CAATCAAAAGCTTCAGC*AAGCAGGAAGGTAC-3' (SEQ ID NO:1) (corresponding to sense nucleotides 977-1008, mutagenesis sites underlined, region of complementarity to primer 3 in italic) and primer 2: 5'- TCG TGT AGA TAA CTA CGA TAC G -3' (SEQ ID NO: 3), corresponding to nucleotides 2050-2071 of pBS. The PfiM I template was amplified with primer 1: 5'-*GCTGAAGCTTTTTGATTG*TAGCGGATCA CTTAG -3' (SEQ ID NO:2) (corresponding to antisense nucleotides 994-962, mutagenesis sites underlined, region of complementarity to primer 1 in italic) and primer 4 (the entire primer 4 was complementary to primer 2). The PCR products were mixed together and cotransfected into bacteria. The Bal I/PfiM I fragment of a plasmid containing the Hind III mutation was then ligated into Ins-pBS that had not undergone PCR amplification. DNA sequencing of the PCR-amplified Hind III/PfiM I region did not reveal any cloning artifacts or polymerase errors.--